1: Biochemistry. 1994 Mar 1;33(8):2104-12. Related Articles, Lir

Identification of a site necessary for allosteric regulation in T4-phage deoxycytidylate deaminase.

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An allosteric inhibitor of dCMP deaminase, dTTP, forms a photolabile covalen bond with T4-phage dCMP deaminase in the presence of UV light at 254 nm. T importance of the methyl group in this process is supported by the findings that dUTP, also an allosteric inhibitor, does not photofix to the enzyme and that tritium is released from [methyl-3H dTTP during the course of the photofixatio That the bond formed is photolabile is demonstrated by the fact that tritium is released by about 10-fold over the amount of nucleotide that is photofixed. The amino acid that covalently binds dTTP in T4-dCMP deaminase was identified a Phe112. On conversion of Phe112 to an alanine by site-directed mutagenesis, there was a dramatic change in the enzyme's response to its allosteric effectors when measured early in the reaction, in that the mutant enzyme was as active as the wild-type even in the absence of dCTP and was only weakly inhibited by dTTP. However, after 10-15% of the substrate had been deaminated, the reactio rate fell off rather markedly, indicating either that an inhibitor was being accumulated on the enzyme or that the enzyme was being irreversibly inactivate with time. That the latter was not the case was shown by the addition of dCTP t the reaction, which restored the rate to that expected when it was present initiall Furthermore, we showed that, consistent with the observed loss of allosteric regulation by dCTP and dTTP, the affinity of the mutant enzyme for dTTP and dCTP as determined by binding studies was greatly reduced relative to the wild-type enzyme.(ABSTRACT TRUNCATED AT 250 WORDS)

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1: Mol Cell Biol. 1986 May;6(5):1711-21. Related Articles, Lir

Sequence and expression of the dCMP deaminase gene (DCD1) (Saccharomyces cerevisiae.

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The dCMP deaminase gene (DCD1) of Saccharomyces cerevisiae has been isolated by screening a Sau3A clone bank for complementation of the dUMP auxotrophy exhibited by dcd1 dmp1 haploids. Plasmid pDC3, containing a 7-kilobase (kb) Sau3A insert, restores dCMP deaminase activity to dcd1 mutant and leads to an average 17.5-fold overproduction of the enzyme in wild-type ce The complementing activity of the plasmid was localized to a 4.2-kb PvuII restriction fragment within the Sau3A insert. Subcloning experiments demonstrated that a single HindIII restriction site within this fragment lies withi the DCD1 gene. Subsequent DNA sequence analysis revealed a 936-nucleotide open reading frame encompassing this HindIII site. Disruption of the open readi frame by integrative transformation led to a loss of enzyme activity and confirm that this region constitutes the dCMP deaminase gene. Northern analysis indica that the DCD1 mRNA is a 1.15-kb poly(A)+ transcript. The 5' end of the transcript was mapped by primer extension and appears to exhibit heterogeneou termini. Comparison of the amino acid sequence of the T2 bacteriophage dCMI deaminase with that deduced for the yeast enzyme revealed a limited degree of homology which extends over the entire length of the phage polypeptide (188 amino acids) but is confined to the carboxy-terminal half of the yeast protein (3 amino acids). A potential dTTP-binding site in the yeast and phage enzymes wa identified by comparison of homologous regions with the amino acid sequences a variety of other dTTP-binding enzymes. Despite the role of dCMP deaminase dTTP biosynthesis, Northern analysis revealed that the DCD1 gene is not subje to the same cell cycle-dependent pattern of transcription recently found for the yeast thymidylate synthetase gene (TMP1).

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